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14. ABSTRACT

This annual report for the Physician Research Training Award focuses on progress in the genetic analysis of circulating hormone refractory prostate cancer micrometastases. As metastatic tissue is often inaccessible in advanced prostate cancer patients, analysis of circulating tumor cells may provide understanding of the biology of hormone refractory prostate cancer as well as chemotherapy resistance. Oligonucleotide array comparative genomic hybridization allows the assessment of genetic changes that may occur in the process of metastasis and chemotherapy resistance. Genomic profiling using this technology will go beyond cell counting, and circumvent technical complexities related to working with RNA. Work performed over the last year has perfected techniques to deal with small amounts of DNA isolated using the Vitatex cell isolation system. Preliminary data suggests that reproducible genomic alterations are observed in the circulating tumor cells isolated from patients with metastatic hormone refractory prostate cancer. During year 3, having ironed out the methodology of pursuing this work, we will ramp up collection of peripheral blood on patients with hormone refractory prostate cancer to isolate circulating tumor cells and perform genetic analyses.

15. SUBJECT TERMS
circulating tumor cells, prostate cancer, chemotherapy resistance, genomic analysis

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Introduction

This project focuses on the genetic analysis of circulating hormone refractory prostate cancer micrometastases with the goal of identifying mechanisms of chemotherapy resistance. Hormone refractory prostate cancer (HRPC) metastatic tissue is difficult to obtain for research, as most metastatic sites are not conducive to biopsy. However, circulating tumor cells (CTC's) have been found in high numbers in patients with metastatic HRPC. CTC's represent an untapped resource for studying the genetics of metastatic HRPC. These cells are easily accessible in the peripheral blood. The purpose of this research is to detect genetic alterations that occur during the development of chemotherapy resistance, to give insight into the mechanisms behind this resistance, and determine potential therapeutic strategies to combat it. To accomplish this, we have refined the techniques needed to isolate CTCs and genomic DNA from those cells, amplify the DNA if necessary, and evaluate genomic alterations using oligonucleotide comparative genomic hybridization (oCGH). Frequently used CTC isolation technologies (e.g. Veridex) do not allow for highly efficient interrogation of DNA because the viable CTCs are not recovered in sufficient purity. We sought to use a technique that would allow us to go beyond CTC enumeration. Our results using the Vitatex technology to capture living CTCs suggest that this approach is feasible and cost efficient. This technology will be incorporated into an upcoming phase II study of second-line chemotherapy for hormone refractory prostate cancer to investigate the "lethal phenotype" of prostate cancer. We hypothesize that the copy number changes could be prognostic and aid in future chemotherapy regimen selection. This report will summarize the progress on this grant and the challenges and obstacles that have arisen and how they will be overcome.

Tasks 1, 2: *Isolation and characterization of circulating micrometastases of chemotherapy naïve and chemotherapy resistant HRPC.*

Procedures and techniques to capture circulating cells using cell-adhesion matrices (CAM) have been continually optimized. This has taken a significant amount of effort and time. The methodology of DNA amplification for CTC DNA was piloted and preliminary data demonstrates the methodology has good fidelity compared with unamplified DNA. In addition, further experiments evaluating genomic changes in CTCs from HRPC patients show promising results, described below.

Most prior studies involving CTCs in prostate cancer patients have been enumeration studies or gene expression studies. Gene expression is dependent on RNA extraction procedures and on environment. Therefore, the disparate published results may be related to minor differences in RNA isolation techniques and the environment of the cells prior to and after isolation. Expression profiles of CTCs may share only limited concordance with cells from the primary tumor and significant variation within and between patients is expected. Genomic profiling will go beyond cell counting, and circumvent technical complexities related to working with RNA.

Currently oligonucleotide comparative genomic hybridization (oCGH) requires 500ng of input DNA. However, the amount of DNA isolated from circulating tumor cells may be less than 500ng. Because the same issue confronts clinical application of array CGH, the Paris/Collins laboratory has been evaluating linear and rolling circle methods for the isolation of DNA from formalin-fixed paraffin-embedded (FFPE) biopsy specimens.[1] Data suggests that it is possible to obtain DNA from paraffin that works very well for array and oligonucleotide CGH and that whole genome amplification (WGA) does not introduce unacceptable copy number artifacts as determined using array CGH. Similar oCGH profiles obtained with unamplified and matching WGA amplified FFPE prostate DNA. Therefore, if necessary, extraction of DNA from circulating tumor cells followed by whole genome amplification should provide sufficient high quality DNA for use with oCGH. FFPE biopsy samples can be treated similarly, if needed. These methodologies were refined over the last year and are now able to be applied to DNA isolated from CTCs.

We have been able to extract on average 7 micrograms of DNA (range 1 μg -16 μg) from isolated cells taken from 20 mL of peripheral blood for use in genomic analysis. A total of 14 patients have been collected to date, and we have been able to isolate CTC DNA from 9 of those patients. Now that technical details have been worked out and preliminary results have been obtained (see below) suggesting that we are able to isolate CTCs using this technology, we are planning on prospectively collecting CTCs in patients enrolling on the phase II component of NCI7347, “A phase I/II study of ixabepilone, mitoxantrone, and prednisone in patients with metastatic hormone refractory prostate cancer previously treated with chemotherapy.” The study will enroll 58 patients, and we expect that, based on previous experience with this isolation technique and prior data regarding the prevalence of CTCs in patients with metastatic HRPC patients, approximately 40 patients will have suitable CTC genomic DNA for analysis.

Tasks 3,4,5,6
Analyze and compare gene signatures of circulating tumor cells to biomarkers previously identified, identify markers of chemotherapy resistance and response in CTC's in HRPC.

During the time period in which we demonstrated that CTC DNA can be isolated from whole blood of prostate cancer patients, the Paris/Collins laboratory switched to the Agilent oCGH platform because it offers comparable data to the BAC arrays, but at a much higher resolution (9kb versus 1.4Mb) and works well with smaller amounts of DNA (500ng).[1] As a result, the Agilent oCGH platform was utilized for the CGH studies.

Data has been generated from 9 samples for which DNA was able to be isolated from CTCs. White blood cells (WBCs) were collected from each patient. Three matched CTC and WBC samples were profiled and in each case the percentage of the genome that was altered in the CTC DNA was larger. These data are presented in Table 1. The frequency of DNA copy number changes observed in the CTCs is shown in Figure 1. Recurrent alterations are being identified in different CTCs from different patients, suggesting that genes may be present at the identified loci that are involved in HPRC pathogenesis. Prospectively collected specimens from a uniformly treated patient population as part of the phase II study described in Task 1 will be analyzed over the next year to elicit statistically meaningful prognostic DNA based biomarkers.

Two of the patients (#8 and #13) had tissue available from their radical prostatectomy (RP) procedure. High volume tumor areas were macrodissected with the assistance of a pathologist, Dr. Jeffrey Simko, and DNA extracted. The RP DNA was profiled on the Agilent arrays and compared to the matched CTC copy number profile. The Kappa score, an indication of the correlation between each of the two profiles, was comparable for each set (Table 2).

To further support the identity of the isolated cells as CTCs, we have collaborated with Dr. Wen-Tien Chen at SUNY Stonybrook who has conducted extensive experiments spiking PC3 (prostate cancer cell line) cells into whole blood of a healthy donor, and demonstrated high efficiency recovery using the CAM Vitatex system. In addition, his laboratory has enumerated CTCs in blood samples from 27 patients with metastatic prostate cancer. The number of CTC recovered in the blood averages over 200 CTC/mL. Currently, we are replicating this work to isolate spiked PC3 cells in whole blood from a healthy volunteer to demonstrate that DNA isolation and oCGH data can be reliably generated, further confirming the robustness of this technology.

More comprehensive oCGH data analysis is currently being undertaken. The above work is being incorporated into a manuscript, in development at this time.

Previous work enumerating CTCs in the blood of patients with prostate cancer done in collaboration with Dr. John Park and Dr. Jorge Garcia has been published (see attached manuscript).

In related work, Dr. Rosenberg is collaborating with Dr. Paris to utilize a library of 44 specimens obtained from patients with HRPC – a unique resource with the potential to be leveraged for the identification of novel genomic pathways associated with castration resistance. Novel pathway identification is a high priority in HRPC. Multiple novel therapeutic agents and strategies are in development creating the major challenge of matching an investigational agent to the biological pathways that are active in a given clinical state of disease. We hypothesize that genome copy number profiles can be used to define the mechanisms of disease in HRPC. Array comparative genomic hybridization (aCGH) is a powerful tool for biomarker discovery and identification of genes involved in cancer progression because it allows high resolution and quantitative detection of copy number aberrations in tumor genome that can be associated with clinical outcome.[2-4] Recurrent deletions and amplifications reveal loci encoding tumor suppressor genes and oncogenes, respectively, and their identification is expedited by using the human genome sequence. More recently, oligonucleotide CGH has allowed for higher resolution copy number profiles. The results from this companion work will increase the informativeness of the results from the study undertaken with CTCs. This upcoming project has been funded by the UCSF Research Evaluation and Allocation Committee.

Task 7

Educational component

Dr. Rosenberg meets regularly with Dr. Small to discuss research and clinical trial design, as well as with Dr. Paris to discuss progress on CTC isolation and characterization. Dr. Rosenberg has been named to the CALGB Genitourinary Oncology Core Committee, and is involved in the decision-making for the new and ongoing clinical trials and translational research of the Committee. Dr. Rosenberg also participates in the weekly Urologic Oncology conference, and bi-weekly Hematology-Oncology conference.

Table 1: Comparing the number of copy number changes in matched CTC and WBC samples

Samples	Percentage of the genome that is aberrant
CTC7	3.21%
WBC7	0.97%
CTC8	2.04%
WBC8	0.60%
CTC13	1.26%
WBC13	0.85%

Table 2. The correlation score for the copy number profiles of matched CTC and radical prostatectomy (RP) samples.

Matched Pairs	Kappa Value
CTC8 and RP8	0.9873
CTC13 and RP13	0.8346

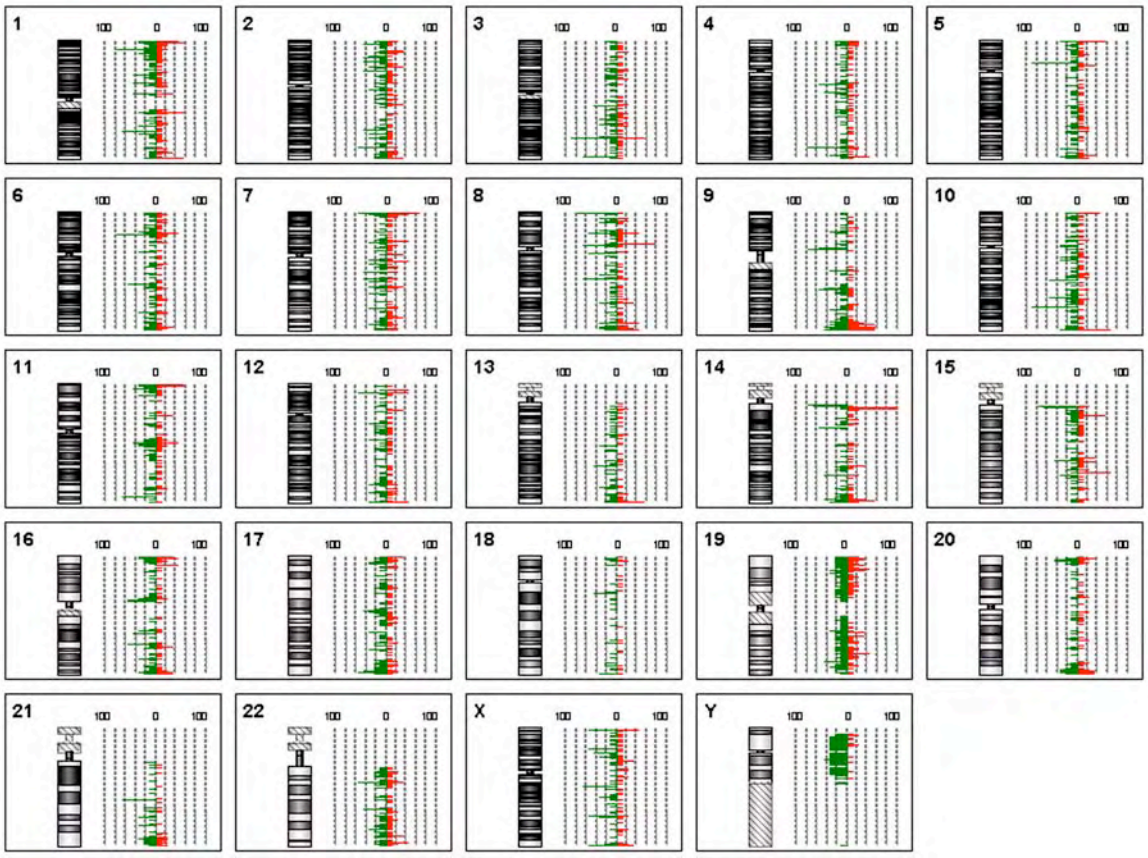

Figure 1. Frequency plot for the nine CTCs is plotted against the chromosomal position. The frequency of gains is shown in red for each chromosome and the frequency of deletions is shown in green, each ranging from 0-100% in 20% intervals. Note the recurrent changes between patients may represent loci associated with HRPC.

Key Research Accomplishments:

- Demonstration that reproducible genomic changes can be observed in CTCs using the Vitatex isolation technology.
- Obtained funding for HRPC tissue-based study to investigate oCGH changes and identify new pathways.

Reportable Outcomes:

"Evaluation and significance of circulating epithelial cells in hormone refractory prostate cancer patients"

[1]Jorge A. Garcia, [2] Jonathan E. Rosenberg, [1] Vivian Weinberg, [1] Janet Scott, [1] Mark Frohlich, [1] John Park, [1] and Eric J. Small [1, 3]

From the Departments of Medicine,[1] University of California, San Francisco, UCSF Comprehensive Cancer Center Biostatistics Core, [2] Department of Urology, [3] UCSF Comprehensive Cancer Center.

British Journal of Urology, 99(3):519-24 (2007).

"Activity of second-line chemotherapy in docetaxel-refractory hormone refractory prostate cancer patients: randomized phase II study of ixabepilone or mitoxantrone and prednisone"

[1] Jonathan E. Rosenberg, [1] Vivian K. Weinberg, [2] W. Kevin Kelly, [3] Dror Michaelson, [4]Maha H. Hussain, [5] George Wilding, [6] Mitchell Gross, [1] Douglas Hutcheon, [1] Eric J. Small, MD

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Cancer, 110(3): 556-63 (2007).

Conclusions:

We have demonstrated that the Vitatex technology can be used to isolate CTCs for genomic analysis. Confirmatory experiments have been conducted by collaborators. High quality DNA is able to be isolated from these cells. oCGH using CTC DNA isolated by the Vitatex system suggests that recurrent genomic alterations are present in CTCs. Specimen collection will continue as part of a prospective clinical trial of HPRC patients. Once sufficient numbers of specimens have been obtained, we will be able to begin to evaluate the genomic alterations associated with CTCs in HRPC in general, and chemotherapy resistance in particular. The Agilent array technology is high resolution allowing the identification of specific genes that may be altered in metastatic and chemotherapy refractory HRPC. Other companion work will evaluate oCGH data in HRPC solid tissue specimens previously collected.

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Urological Oncology

The urological oncology section is relatively long this month, and this reflects the many high-quality manuscripts we receive. When you consider our relatively high rejection rate, you will understand just how many papers on this topic are submitted. The high quality of oncology papers is clear in this month's section. You will also notice that all but one of them are on prostate cancer, and the reason for this is similar to that mentioned above, as this topic is, as might be expected, the most commonly submitted in this section. However, I am only too happy to reassure readers, and those primarily interested in other types of urological cancer, that the imbalance in this month's section is not a permanent fixture.

Evaluation and significance of circulating epithelial cells in patients with hormone-refractory prostate cancer

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OBJECTIVE

To determine the feasibility of using flow cytometry fluorescence-activated cell sorting (FACS) analysis for detecting circulating epithelial cells (CECs) in patients with hormone-refractory prostate cancer (HRPC), and to determine whether CECs can be used to predict survival in these patients.

PATIENTS AND METHODS

Several prognostic models that include routinely used clinical and laboratory variables for predicting survival in men with HRPC have been reported; the presence of CECs measured by reverse transcriptase-polymerase chain reaction for prostate-specific antigen (PSA) in patients with HRPC is an independent prognostic factor for survival. CECs detected by FACS analysis correlate with advanced stage and poor survival outcome. A retrospective study was conducted to assess the presence of CECs by FACS analysis in metastatic HRPC patients initiating systemic chemotherapy with a taxane-based regimen. The association between clinical variables previously described and the presence of CECs along with the effect of the magnitude of CECs on survival was calculated, in 41 patients with HRPC, all of whom had peripheral blood collected for FACS analysis.

RESULTS

Except for four patients, all those with metastatic HRPC had detectable CECs. Among these patients, the number of CECs/mL was correlated with age, serum PSA level and serum alkaline phosphatase (ALP). Higher serum levels of PSA and ALP predicted a poor survival outcome. Similarly, patients with ≤1.8 CECs/mL had a significantly longer survival than those with more CECs/mL ($P = 0.02$). With a median follow-up of 15.4 months, the median overall survival for all patients was 18.4 months.

CONCLUSIONS

The presence of more CECs in patients with metastatic HRPC was associated with a poorer survival outcome; levels of ≥1.8 CECs/mL were associated with a shorter survival in patients with metastatic HRPC.

KEYWORDS

hormone-refractory prostate cancer, circulating epithelial cells, flow cytometry, fluorescence-activated cell sorting, chemotherapy

INTRODUCTION

Prostate cancer remains the most common cancer among men in the USA, accounting for >32% of all male malignancies. It is estimated that >234 000 men will be diagnosed with prostate cancer during 2006, and 27 350 will die from the disease. Virtually all deaths are due to the development of hormone-refractory prostate cancer (HRPC) [1]. Several prognostic models predictive of survival in men with HRPC have been reported [2–5]. Numerous reports have suggested that early-stage cancers have the potential to begin shedding cancer cells into the circulation early in their development. Unfortunately, the natural history of these cells, their ability to establish metastases, and their role in disease recurrence remains unclear. Detection of micrometastases, or circulating tumour or epithelial cells (CECs) has become an attractive technique that can be used to assess the prognosis in patients with cancer. Several authors showed that levels of CECs in patients parallel the tumour burden and response to therapy [6–12]. Indeed, the number of circulating tumour cells before treatment was recently found to be an independent predictor of progression-free and overall survival in patients with metastatic breast cancer [13]. CECs can be detected in 0–72% of patients with prostate cancer that is clinically organ-confined and in 25–100% of patients with distant metastatic disease. The presence of CECs at the time of primary therapy has also been associated with early disease failure and poor long-term outcome [14,15]. Various groups also showed that the presence of CECs measured by reverse transcriptase (RT)-PCR for PSA in patients with HRPC receiving cytotoxic chemotherapy correlated with survival outcome [16–20]. Positive RT-PCR for PSA is an independent prognostic factor for survival in men with HRPC [21]. Halabi *et al.* [22] confirmed that RT-PCR for PSA is a statistically significant predictor of overall survival for patients treated once with previous hormonal therapy.

RT-PCR for CECs has several limitations; the lack of specificity coupled with the lack of standardization of RT-PCR techniques has prevented this test from achieving widespread use. By contrast, fluorescence-activated cell sorting (FACS) analysis allows the detection of antigens in a heterogeneous mixture of cells, and offers several advantages over immunohistochemistry and RT-PCR. Cell sorting is easy to do and enables a high throughput of samples, quantification of results, and isolation of subpopulations of cells. The feasibility of using FACS assays for detecting micrometastases was reported in several cancers [12,13,20,23]. Compared with normal individuals there are significantly more CECs identified by FACS analysis in patients with prostate cancer. Also, the presence of CECs in patients with advanced prostate cancer appears to correlate with survival [24–27]. Unfortunately, limited sample sizes and the lack of clinical correlation make these results insufficient to assess the true clinical utility of this test. We report the results of a retrospective pilot analysis that evaluated patients with HRPC undergoing cytotoxic therapy, to determine the utility and feasibility of FACS analysis for detecting CECs, their change over time, and to assess whether or not the presence and number of CECs identified by FACS analysis was a predictor of outcome in men with HRPC.

PATIENTS AND METHODS

This was a retrospective study of 41 consecutively treated patients with metastatic HRPC who were starting systemic chemotherapy. All patients had peripheral blood collected before starting systemic cytotoxic chemotherapy with a taxane-based regimen. Subsequently, blood was collected at the start of each cycle of chemotherapy until therapy was discontinued. All 41 patients have had, and subsequently discontinued, second-line hormonal manipulations before entry to the present study. There were no uniform criteria applied for either the discontinuation of second-line hormonal therapy or the subsequent institution of systemic chemotherapy. For patients with measurable disease, progression was defined as a ≥20% increase in the sum of the longest diameter of target lesions or the appearance of one or more new lesions, as for the Response Evaluation Criteria in Solid Tumors system [28]. Patients with no measurable disease were required to have a positive bone scan and elevated PSA level. PSA evidence for progressive prostate cancer consisted of a PSA level of ≥5 ng/mL, which had risen above the minimum of the nadir and baseline on at least two successive occasions, at least 2 weeks apart. Response to therapy was assessed by Consensus Criteria [29]. There were no uniform criteria applied for the minimum or maximum number of peripheral blood collections required while patients were receiving systemic chemotherapy.

For the isolation and enumeration of CECs, blood samples were drawn into 10-mL EDTA-Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) to which a cell preservative was added [30,31]. Samples were maintained at room temperature and processed within 24 h after collection. All FACS analyses were performed at a central laboratory within our institution. For each sample the lymphocyte/monocyte fractions were separated using Ficoll-Hypaque density-gradient centrifugation. A positive-selection pre-enrichment step was used, by incubating the lymphocyte/monocyte fractions of each sample with ferrofluid particles coated with MJ37 (an anti-epithelial surface antigen encoded by the EGP2 or GA-733-2 gene, EpCAM) monoclonal antibody. The anti-EpCAM (EBA-1) antibody that recognises epitopes different from MJ37 was also added. The sample tube was then subjected to a magnetic field for 45 min in a magnetic separator and the sample blood aspirated from the tube. The sample tube was removed from the magnet and cells remaining in the tube were resuspended in 2 mL of cell buffer. The re-suspended cells were transferred to one 12 × 75 mm polystyrene tube and subjected to magnetic separation for 5 min. The fluid material in the tube was aspirated and the pellet of cells was re-suspended in 150 μL of cell buffer. The antibody CD45 PerCP-Cy5.5 and a nucleic acid dye (ProCOUNT, Becton Dickinson) were added (20 μL). Fluorescently labelled monoclonal antibodies specific for leukocytes (CD45 PerCP-Cy5.5) and ECs (MJ-37 and EBA-1) are used to distinguish ECs from leukocytes. Samples were incubated in the dark for 15 min, and then fixed by adding 350 μL of 1% paraformaldehyde. Subsequently, samples were transferred to a TruCOUNT tube (Becton Dickinson) and then run on a FACS Calibur (Becton Dickinson) with four-colour option, until 35 000 bead events were acquired. Each sample was acquired with a threshold on both EpCAM (EBA-1) and nucleic acid dye (ProCOUNT). Circulating tumour cells were defined as nucleated cells, which are

Characteristic (n in sample)	Value
Median (range) age, years (40)	70.1 (44–89)
Median (range) initial PSA level, ng/mL (40)	50.2 (0.9–3019)
n (%) with PSA level of:	
<10.0	10 (25)
10.0–100.0	17 (42)
>100.0	13 (33)
Mean (SD) ALP, IU/L	184.8 (211.8)
Median (range)	111.0 (58.0–1160)
Mean (SD) haemoglobin, g/dL (40)	12.7 (2.0)
Median (range)	12.7 (9.1–18.4)
n (%) with <12.0	11 (28)
Mean (SD) LDH, U/L (40)	174.4 (64.1)
Median (range)	152.5 (129–470)
ECOG performance status, n (%)	
0	26 (64)
1	10 (24)
2	5 (12)
Gleason sum, n (%)	
5–6	7 (17)
7	21 (51)
8–9	13 (32)
No. of previous systemic therapies	
0	1 (2)
1	15 (37)
2–3	21 (51)
4–5	4 (10)
Median blood volume/sample, mL	20
Mean (SD, range) volume sampled	128.1 (197.5, 0–1005)
Median (range) CECs/mL	1.8 (0–55.8)
Mean (SD)	6.97 (10.66)
N (%) with CECs/mL of	
0	4 (10)
0.1–5.0	20 (49)
>5.0–15.0	10 (24)
>15.0–30.0	6 (15)
>30.0	1 (2)

TABLE 1
Patient characteristics and CEC counts for the 41 men in the study

simultaneously EpCAM-positive, ProCOUNT-negative and CD45-negative [32,33].

The data analysis was primarily descriptive; each patient's disease characteristics at the time of entering the trial were collected, including PSA level, Gleason score, extension of metastatic disease, details of previous therapy, and laboratory variables. Descriptive statistics were used to characterize the entire patient sample. Subsets were compared using Fisher's exact test for categorical variables, ANOVA methods for continuous variables and the nonparametric Mann–Whitney *U*-test to compare distributions. The association between continuous variables was estimated by the Spearman rank correlation coefficient. The Kaplan–Meier product-limit method was also used to estimate the probability of survival, with the log-rank test used to compare distributions of subsets. Survival was measured from the start of chemotherapy until either death or the date of last contact. Multivariate analyses were done using Cox proportional-hazards model to identify independent predictors of survival. A forward stepwise approach was used, with significance determined by the likelihood-ratio test. Coefficients for significant predictors were tested using the Wald statistic.

RESULTS

FACS analysis data from 41 patients with metastatic HRPC who initiated systemic taxane-based chemotherapy at our institution between 1999 and 2001 were included; their characteristics are summarized in Table 1. All patients had radiographic evidence of metastatic disease in either soft tissue, bone or both (39%, 61% and 22%, respectively). The initial median (range) PSA level for all evaluable patients was 50.2 (0.9–3019) ng/mL; 30% had PSA levels of <20 ng/mL and 80% had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1. The median (range) alkaline phosphatase (ALP) level was 111 (59–1160) U/L and the median haemoglobin level was 12.7 (9.1–18.4) g/dL. Overall, 51% of patients had a Gleason score of 7, while in 32% it was 8–10. As defined by the consensus criteria, all patients had castrate testosterone levels. More than half of the patients (61%) had received at least two previous systemic therapies that included androgen deprivation, immunotherapy on a clinical trial, and secondary hormonal manoeuvres with agents such as antiandrogens, oestrogens and ketoconazole.

The number of peripheral blood collections in the patients varied; half (51%) had only one collection for FACS analysis just before starting chemotherapy, 49% had more than one collection, and 15% had 7–15 collections. Most patients (66%) had 20 mL of blood collected, and no patient had <9.5 mL collected. When analysed by the volume of blood obtained (<20 vs 20 mL) for the first collection, there was no difference in the number of CECs/mL (data not shown).

There were no CECs in the peripheral blood in only four patients; all four had bone metastases only and their Gleason score was 7 in two and 8 in two. There were no significant differences between this small subset and the entire cohort. Overall, 49% of patients had 0.1–5.0, 24% had >5–15, 15% had >15–30 and 2% had >30 CECs/mL.

Among all patients the number of CECs/mL obtained at the time of first collection was significantly correlated with PSA level, age (inversely) and ALP levels, with a Spearman rank correlation, r, of 0.53 ($P < 0.001$), –0.33 ($P = 0.04$) and 0.38 ($P = 0.02$), respectively. At the time of the first collection the association was strongest between the number of CECs/mL and PSA level ($P = 0.01$). If a patient had a PSA level of <20 ng/mL, then 83% also had <1.8 CECs/mL (the median). There was more variability in range for the CECs/mL if the patient had a PSA level of >20 ng/mL but most (61%) had >1.8 CECs/mL. The decreasing concentration of CECs with increasing age

reflects that those patients aged <65 years (the lower age quartile) more often had more than the median value of 1.8 CECs/mL (67%), whereas those aged ≥75 years usually had fewer than the median (67%). Of all patients, 80% with ALP levels of >200 U/L (the upper quartile) had >1.8 CECs/mL ($P = 0.02$). By contrast, patients with ALP levels of <110 U/L (the median) were more likely to have <1.8 CECs/mL (65%), resulting in the increasing correlation. For the first collection there was no association between the concentration of CECs and lactate dehydrogenase (LDH), haemoglobin, ECOG performance status or the number of previous therapies. Using the overall median (1.8 CECs/mL) to dichotomize the patients, those with ≤1.8 CECs/mL had significantly longer survival than those with >1.8 CECs/mL. The median survival of patients with metastatic HRPC with >1.8 CECs/mL was 13 months; that for patients with ≤1.8 CECs/mL has not been reached ($P = 0.02$; Fig. 1). Moreover, there were no associations between changes in serum PSA level, serum ALP and the number of CECs/mL with disease response while on therapy. Nevertheless, when several measurements were available, there were often similar patterns over time for CECs/mL, PSA and ALP levels; Fig. 2 shows an example of this relationship.

Additional univariate analyses indicated that having a PSA level of <20 ng/mL, ALP of ≤110 U/L, a Gleason score of ≤7 or having had only one previous therapy resulted in a more favourable survival outcome ($P = 0.01$, 0.03, 0.05 and 0.02, respectively). Multivariate analyses using a Cox proportional-hazards model were used to identify significant independent predictors of survival from among those significant factors determined by univariate methods. This included CECs/mL (≤1.8 vs >1.8), PSA and ALP levels, Gleason score (≤7 vs 8–10) and the number of previous therapies (1 vs >1). Both CECs/mL and the number of previous therapies were independent predictors of survival (likelihood-ratio test, $P = 0.02$ for each factor; Table 2). The median survival for all patients was 18.4 months; 19 of the 41 patients died, all within 20 months of starting chemotherapy, and 10 survived beyond that time for up to 65 months from diagnosis.

FIG. 1. Overall survival vs CECs/mL in patients with HRPC.

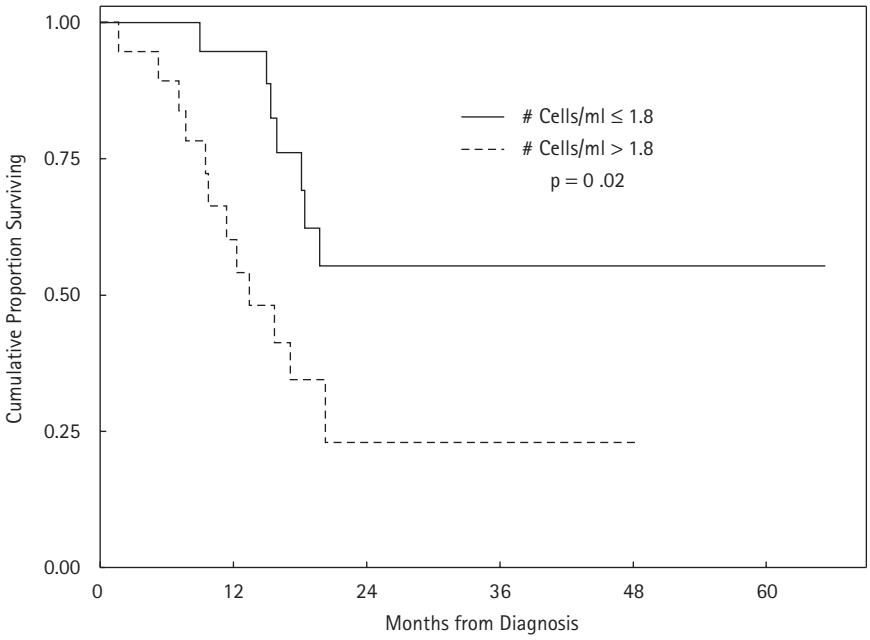
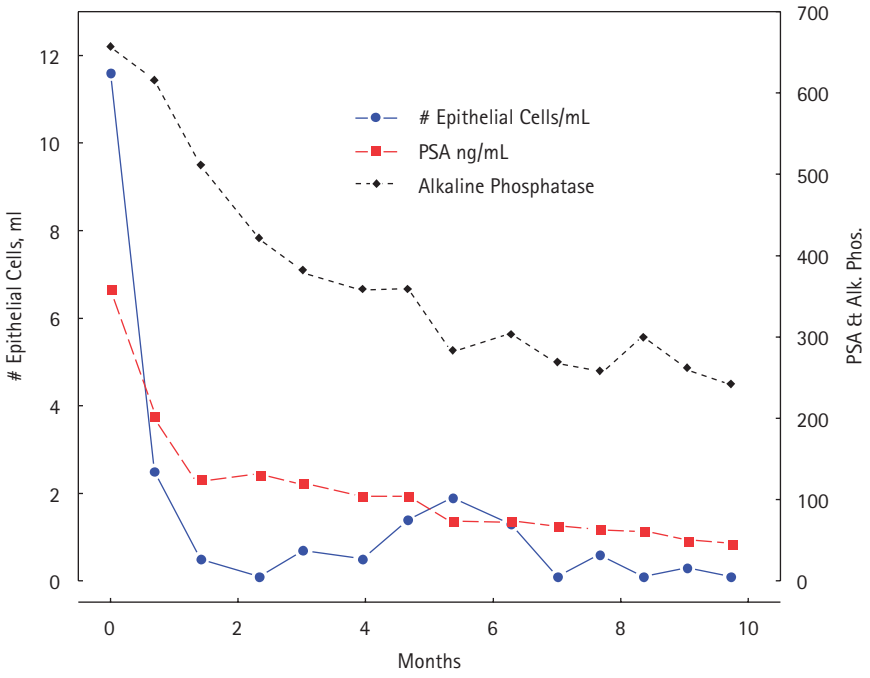


FIG. 2. The relationship between PSA and ALP levels, and CECs in patients with HRPC undergoing palliative systemic cytotoxic chemotherapy. CECs, PSA and ALP levels were recorded at different times during the patient's chemotherapy treatment.



DISCUSSION

This retrospective analysis evaluated the feasibility of using FACS analysis for detecting CECs in patients with HRPC; we also evaluated the correlation between the level of CECs and other clinical variables, e.g. PSA, ALP, LDH, and haemoglobin, all clinical features previously shown to affect the outcome in such patients.

Although the analysis was limited by being retrospective and including relatively few patients, CECs were present in the vast majority of the patients. There was no reference point to relate the time of collection

TABLE 2 Univariate and multivariate analysis; predictors of survival

Variable	Univariate; log-rank test P	Multivariate; independent predictors of survival		
		Likelihood ratio, P	Hazard ratio (95% CI)	Wald, P
PSA, <20 vs ≥20	0.01			
CEC/mL, ≤1.8 vs >1.8	0.02	0.02	3.2 (1.2–8.3)	0.02
Previous therapy, 1 vs >1	0.02	0.02	3.3 (1.1–9.4)	0.03
ALP, ≤110 vs >110	0.03			
Gleason score, ≤7 vs 8–10	0.05			

with the course of disease, and hence any of the differences noted in this analysis only reflect the data at one point in time and not necessarily a common point for all patients. Therefore, these results require validation in a prospective trial, and cannot be universally applied to all patients with HRPC.

In the present analysis there were very strong correlations between the concentration of CECs, and serum PSA and ALP levels; hence, >1.8 CECs/mL, a serum PSA level of ≥20 ng/mL and serum ALP levels of >110 U/L (the median values) were each strong predictors of a poorer outcome ($P = 0.02$, 0.01 and 0.03, respectively). Unfortunately, this limited study could not define an association between changes in serum PSA and ALP levels, and number of CECs/mL, with disease response. However, it was suggestive that the pattern of CECs/mL measured over time appeared to mirror the PSA pattern (with an increase or plateau) in an individual patient while on chemotherapy. Also, when several measurements were available, there were often similar patterns over time for CECs/mL and ALP levels. This reflects the correlation between these factors that was identified at the initial collection, and might suggest that in addition to clinical symptoms, serum PSA level, and imaging studies, CECs could potentially be used for predicting and assessing the response to systemic chemotherapy in patients with HRPC.

Similar to our data, Moreno *et al.* [34] reported their experience using FACS analysis for evaluating CECs in patients with advanced prostate cancer. Among their 26 patients with HRPC, the presence of ≥5 CECs/7.5 mL of blood was a strong predictor for survival outcome (hazard ratio 7.18, $P = 0.002$). After a multivariate Cox analysis the presence of CECs was of borderline significance in a model for predicting the survival in patients with HRPC (hazard ratio 4.18, $P = 0.056$). Similarly, their study showed that patients with <5 CECs/7.5 mL of blood had a median overall survival time of 2.5 years, compared with 0.5 years in patients with >5 CECs/7.5 mL ($P = 0.003$).

In the present study there were similar associations between the number of CECs/mL and overall survival. We also dichotomized the patient sample based on the overall median number of CECs/mL. With a median follow-up of >36 months, the overall median survival for all metastatic patients with >1.8 CECs/mL was 13 months, and the median for patients with ≤1.8 CECs/mL, overall or with metastases, has not been reached ($P = 0.02$). Our multivariate analysis also indicated that CECs/mL and the number of previous therapies (which probably represents the extent of the disease process, and later stages in treatment) were each independent predictors of survival ($P = 0.02$ for each).

In summary, we showed that in addition to previously described clinical variables, measuring CECs in patients with HRPC can be used as a prognostic tool to predict the outcome. Having more CECs/mL appears to correlate with shorter survival in patients with metastatic HRPC. Our findings, combined with the results from others, suggest that CECs might be relevant and could be used to predict the outcome in patients with HRPC. Future clinical trials with chemotherapy or novel therapeutics in patients with HRPC should consider the prospective collection of peripheral blood for CEC analyses.

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Abbreviations: **HRPC**, hormone-refractory prostate cancer; **CEC**, circulating epithelial cell; **RT**, reverse transcriptase; **FACS**, fluorescence-activated cell sorting; **ALP**, alkaline phosphatase; **ECOG**, Eastern Cooperative Oncology Group; **LDH**, lactate dehydrogenase.

Activity of Second-Line Chemotherapy in Docetaxel-Refractory Hormone-Refractory Prostate Cancer Patients

Randomized Phase 2 Study of Ixabepilone or Mitoxantrone and Prednisone

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BACKGROUND. This randomized, noncomparative, multicenter, clinical trial evaluated ixabepilone or mitoxantrone/prednisone (MP) as second-line chemotherapy for taxane-refractory, hormone-refractory, prostate cancer (HRPC).

METHODS. Patients with HRPC that progressed during or within 60 days of cessation of taxane chemotherapy were randomly selected with equal probability to ixabepilone 35 mg/m^2 intravenously every 3 weeks, or mitoxantrone 14 mg/m^2 intravenously every 3 weeks and prednisone 5 mg orally twice daily. Treatment continued until progression or toxicity; crossover was allowed.

RESULTS. Forty-one patients were accrued to each arm of the study. The median number of cycles administered for each arm was 3. Median survival from protocol entry was 10.4 months with ixabepilone and 9.8 months with MP. Prostate-specific antigen (PSA) declines of $\geq$50% were observed in 17% of ixabepilone (95% CI, 7-32) and 20% of second-line MP patients (95% CI, 9-35). Partial responses were observed in 1 of 24 ixabepilone and in 2 of 21 MP patients with evaluable measurable disease. Median duration of second-line ixabepilone and MP treatment was 2.2 months and 2.3 months, respectively. For third-line crossover treatment, PSA declines of $\geq$50% were observed in 3 of 27 ixabepilone-treated and 4 of 15 MP-treated patients. Prior taxane response was associated with an increased likelihood of second-line ixabepilone or MP response. Low baseline lactate dehydrogenase and absence of visceral metastases independently predicted improved survival. The most common grade 3/4 toxicity associated with second-line treatment was neutropenia (54% of ixabepilone patients and 63% of MP patients).

CONCLUSIONS. Ixabepilone and MP had modest activity as second-line chemotherapy for docetaxel-refractory HRPC. The median survival for the entire cohort treated in this study was 9.8 months. ***Cancer* 2007;110:556–63.** © *2007 American Cancer Society.*

KEYWORDS: prostate cancer, taxane, hormone, refractory, ixabepilone, mitoxantrone, prednisone, second-line therapy.

Chemotherapy for taxane-refractory, hormone-refractory, prostate cancer (HRPC) is effective at prolonging survival and palliating symptoms. Two large phase 3 studies demonstrated that first-line docetaxel chemotherapy is associated with an improvement in median survival compared with mitoxantrone/prednisone (MP).[1,2]

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Nearly all HRPC patients eventually progress during or after taxane-based treatment. Many patients have a good performance status and wish additional treatment. No standard chemotherapy exists for second-line treatment of patients with HRPC after progression on taxane-based therapies, although the community de facto standard has become MP.

The natural history of taxane-refractory (TR) HRPC has not been prospectively defined. Although second-line chemotherapy trials have been reported in HRPC, these trials are difficult to interpret because of heterogeneity of patient populations. Most importantly, those trials did not restrict enrollment to overtly TR-HRPC.

Resistance to taxanes appears mediated by tubulin mutation and multidrug resistant (MDR) gene overexpression. The epothilones are a new class of nontaxane tubulin polymerization agents whose cytotoxic activity has been linked to stabilization of microtubules, bypassing known taxane-resistant mechanisms.[3,4] Ixabepilone (Bristol-Myers Squibb, New York, NY) is a semisynthetic analog of epothilone B that blocks the mitotic phase of the cell cycle. It is a highly potent cytotoxin, and preclinical data demonstrate noncross-resistance with taxanes. Ixabepilone has demonstrated antitumor activity as first-line chemotherapy in patients with metastatic HRPC.[5,6]

The preclinical data indicating noncross-resistance of ixabepilone with taxanes, the front-line activity of ixabepilone in HRPC, and the lack of prospective data regarding MP as second-line chemotherapy provided the rationale for a randomized, noncomparative, phase 2 study in TR-HRPC. This study randomly assigned patients with TR-HRPC to either single-agent ixabepilone or the perceived community standard, MP.

MATERIALS AND METHODS

Study Design

This study was a multicenter, randomized, noncomparative phase 2 study. Patients were randomly assigned with equal probability to either MP or ixabepilone. The primary endpoint was the frequency of $\geq$50% PSA declines with each second-line regimen. Secondary endpoints included safety, response duration, time to progressive disease, third-line (post-crossover) activity of each regimen, and overall survival.

Eligibility Criteria

All patients had histologically confirmed metastatic prostate cancer. Patients were required to have progressive disease despite castrate testosterone levels and at least 2 cycles of taxane-based chemotherapy, with disease progression documented during or within 60 days of completing taxane-based chemotherapy. For patients with measurable disease, progression was defined by RECIST criteria.[7] For patients without measurable disease, a positive bone scan and elevated PSA greater than 5 ng/mL were required. PSA evidence for progressive prostate cancer was defined by Consensus Criteria.[8]

All patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and $\leq$grade 1 neuropathy (Common Toxicity Criteria, version 2.0). Hormonal therapy other than luteinizing hormone-releasing hormone (LHRH) agonists was not allowed within 4 weeks of trial enrollment (6 weeks for bicalutamide or nilutamide). Treatment with a corticosteroid as part of first-line chemotherapy was discontinued over 10–14 days before enrollment. Any radiation therapy or radiopharmaceutical treatment must have been completed more than 4 weeks and 8 weeks before enrollment, respectively. All patients were required to have a cardiac ejection fraction greater than the institutional lower limit of normal. Patients were excluded for significant cardiovascular disease including congestive heart failure (New York Heart Association [NYHA] class III or IV), active angina pectoris, or myocardial infarction within 6 months before enrollment. Patients with known active brain metastases were excluded. Required laboratory values included testosterone $<$50 ng/dL; creatinine $<1.5 \times$ upper limits of normal (ULN) or calculated creatinine clearance $>$40 mL/min; alanine aminotransferase (ALT) and aspartate transaminase (AST) $<3 \times$ ULN; granulocytes $>1500/mm^3$; platelets $\geq 100{,}000/mm^3$; total bilirubin $<1.5 \times$ ULN; and, if no measurable disease, a PSA $\geq$5 ng/mL.

This clinical trial was sponsored by the Cancer Therapy Evaluation Program of the National Cancer Institute and approved by the review boards of each participating institution. All patients provided written informed consent.

Randomization and Treatment Plan

Eligible patients were randomly selected by the coordinating center statistician with equal probability to receive either ixabepilone or MP. Allocation to a treatment arm was concealed until the patient was enrolled. Patients were stratified by performance score (0 vs 1-2) and study site, and they were randomly assigned from within each stratum. Treatment assignment was balanced after every 4 patients within each stratum.

Ixabepilone 35 mg/m^2 was administered intravenously over 3 hours every 21 days. Patients were

premedicated with H1- and H2-blockers before ixabepilone infusion to prevent hypersensitivity reactions related to Cremophor EL diluent (BASF Group, Ludwigshafen, Germany) Corticosteroids were used with subsequent cycles for prior grade 2-4 hypersensitivity reactions to ixabepilone. Mitoxantrone 14 mg/m^2 was administered intravenously every 21 days with prednisone 5 mg orally twice daily. Treatment for all patients was continued until disease progression or unacceptable toxicity occurred. Myeloid growth factors were administered according to American Society for Clinical Oncology (ASCO) guidelines.[9] Patients underwent imaging with chest s-ray, bone scan, and computed tomography (CT) or magnetic resonance imaging (MRI) of the abdomen and pelvis at baseline and after every 3 cycles. Electrocardiogram and multiple gated-acquisition (MUGA) scan or echocardiogram were obtained at baseline and repeated every 3 cycles for MP patients. Imaging studies were obtained at the time of crossover.

Dose Modifications

Dose modifications were made according to maximal toxicity. Doses were reduced for Day 1 neutrophil count $<1500/m^3$ or platelet count $<100,000/m^3$, $\geq$grade 3 nonhematologic toxicity, grade 4 neutropenia lasting for more than 7 days, grade 4 neutropenia and fever, and nadir platelet count $<25,000$. Ixabepilone dose was reduced by 5 mg/m^2, and mitoxantrone dose was reduced by 2 mg/m^2 for each dose reduction. Grade 2 neurotoxicity of any duration and grade 3 neurotoxicity lasting $\leq$7 days required dose reduction. Recurrent grade 3 neurotoxicity, grade 3 neurotoxicity of $>$7 days duration, or grade 4 neurotoxicity required discontinuation of treatment. Patients were removed from protocol therapy for a treatment delay greater than 3 weeks or recurrence of the same grade $\geq$3 toxicities despite 2 dose reductions.

Crossover Therapy

Patients who progressed after at least 2 cycles of protocol treatment or who stopped treatment for toxicity or other medical reasons were eligible to receive the alternate treatment. For patients initially treated with MP, prednisone was tapered over 10–14 days before starting ixabepilone.

Statistical Considerations

This was a noncomparative randomized phase 2 study to assess safety and efficacy of 2 treatment regimens, ixabepilone and MP, as second-line therapy for metastatic TR-HRPC patients. The primary endpoint was the frequency of PSA declines $\geq$50% with second-line therapy, confirmed with 2 consecutive measurements. Response to therapy was determined for each patient by using PSA declines for nonmeasurable disease, and RECIST criteria for measurable disease, bone scans, and nontarget lesions.[7,8] For each treatment arm, a $\geq$50% PSA decline in at least 25% of patients was considered promising and worthy of further investigation. Accrual of 40 patients to each treatment arm was sufficient to detect a 25% response proportion compared with a null hypothesis of $\leq$10%. A statistical level of significance of 0.04 for a directional test and power of 0.82 was assumed to test this hypothesis. Secondary endpoints included response duration, time to PSA progression, overall survival, frequency of toxicity, and frequency of response to third-line (crossover) treatment.

Comparability of the 2 treatment subsets was evaluated by using Fisher exact test for categorical variables (eg, Gleason score), Student *t* test for continuous variables (eg, lactate dehydrogenase [LDH]), and the Mann-Whitney test for distributions (eg, PSA). The effect of prior taxane response on second-line treatment response was analyzed by using the Mantel-Haenszel tests of association and homogeneity stratified by the second-line therapy.[10] Duration of time to progression and overall survival were calculated from the start of second-line therapy with the Kaplan-Meier product-limit method.[11] Comparisons of a difference in distributions between subsets were performed by using the log-rank test.[12] Cox proportional hazard model was used to identify independent disease features of overall survival for the entire sample.[13] Variables predictive of overall survival based on the log-rank test were considered in building a model. A forward stepwise approach was used with the likelihood ratio test to determine significant independent predictors of survival.

RESULTS

Patient Characteristics and Disposition

Between February 2003 and June 2005, 86 patients were entered at 6 participating centers. Four patients who never started protocol therapy were not included in the analysis, thus 82 patients were evaluable. Forty-one patients were randomly assigned to each treatment arm (Fig. 1). Patient baseline characteristics are detailed in Table 1. Both arms were balanced. All patients who received any protocol chemotherapy were included in evaluations of response and toxicity.

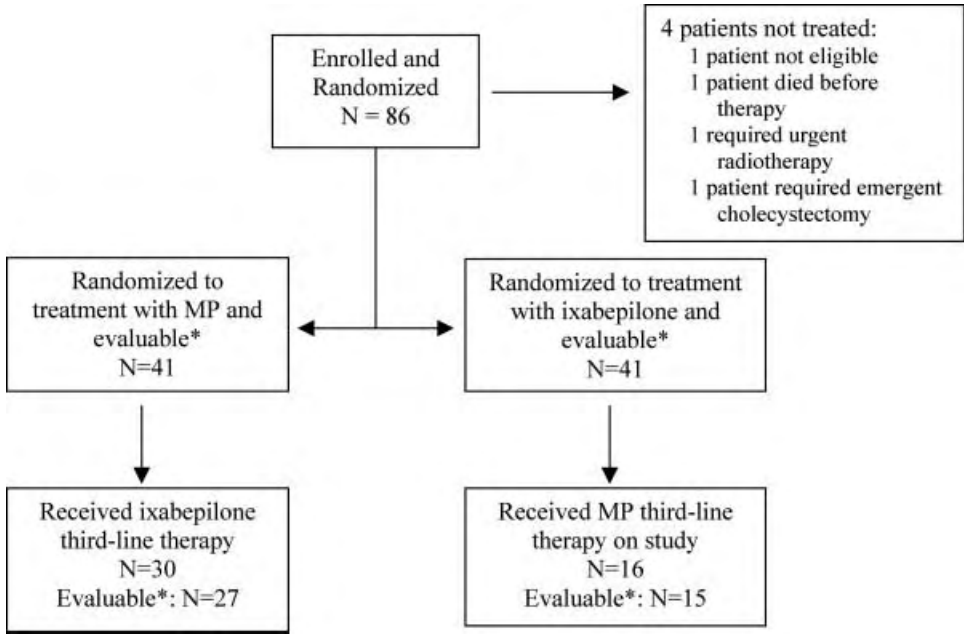

FIGURE 1. Patient Disposition. *Received at least 2 cycles of therapy.

TABLE 1
Baseline Patient Characteristics

2nd Line treatment	Ixabepilone n = 41	MP n = 41
Median age, y (range)	66.5 (51–87)	69 (52–84)
ECOG PS		
0	15 (37%)	15 (37%)
1–2	26 (63%)	26 (63%)
Prior therapy		
Radiation (RT)	10 (24%)	7 (17%)
Prostatectomy (RP)	16 (39%)	15 (37%)
RP+RT	2 (5%)	5 (12%)
Other	13 (32%)	14 (34%)
Median PSA, ng/mL (range)	141 (4–17,995)	113 (7–1587)
Gleason score	n = 37	n = 38
Range	5–10	5–10
5–6	14%	11%
7	32%	18%
8–10	54%	71%
Median LDH, IU/L (range)	266 (103–2291)	273 (101–3065)
Median alkaline phosphatase, U/L (range)	126 (58–1432)	156 (45–664)
Median hemoglobin, g/dL (range)	11.7 (8.8–14.0)	12.2 (8.9–14.7)
Mean No. prior taxane chemotherapy cycles (range)	5.6 (2–25)	6.8 (2–17)
Prior chemotherapy		
Docetaxel-based	18 (45%)	18 (47%)
Docetaxel/estramustine-based	22 (55%)	20 (53%)

Second-Line Study Treatment

A median of 3 cycles of ixabepilone (range, 1 to 22 cycles) and 3 cycles of MP (range, 1 to 12 cycles) were administered as second-line treatment. Thirty-two percent of ixabepilone patients and 27% of MP patients received at least 5 cycles of therapy. Treatment with ixabepilone was discontinued in 7 patients for toxicity, 1 for withdrawal of consent, and 33 patients for disease progression (23 for PSA progression, 6 for objective progression, 1 for both PSA and objective progression, and 4 for clinical and/or symptomatic progression that required additional

TABLE 2
Response to Second-line Therapy

	2nd-Line Ixabepilone no. (%)	2nd-Line MP no. (%)
Evaluable patients	41	41
Confirmed PSA decline ≥50%, 95% CI	7 (17, 7–32)	8 (20, 9–35)
Unconfirmed PSA decline ≥50%	1 (2)	—
Objective disease responses		
Measurable disease	30	23
Evaluable patients*	24	21
Partial response (RECIST)	1	2

* Received at least 2 cycles.

therapy). Treatment with MP was discontinued in 4 patients for toxicity and in 36 patients for disease progression (28 for PSA progression, 6 for objective progression, 2 for both PSA and objective progression). One MP patient died on study of unrelated causes.

Response

Of 41 patients treated with second-line ixabepilone, 7 had a confirmed ≥50% PSA decline (17%; 95% CI, 7-32; Table 2). One additional patient had an unconfirmed ≥50% PSA decline. The median time to a ≥50% PSA decline was 6 weeks (range, 3–14 weeks). Twenty-four patients treated with at least 2 cycles of second-line ixabepilone had measurable disease, and, of these, 1 (4%) patient had an objective partial response in addition to a PSA response. The median time to PSA progression on ixabepilone was 2.2 months, and the median duration of response was 3.8 months (range, 2.8–22.3 months). Three confirmed responders discontinued treatment for toxicity (motor neuropathy, atrial arrhythmia, and grade 2 infusion-site reaction), and 4 confirmed responders discontinued because of progressive disease.

Of the 41 patients treated with second-line MP, 8 had a confirmed ≥50% PSA decline (20%; 95% CI, 9-35; Table 2). For responders, the median time to a ≥50% PSA decline was 7 weeks (range, 3–19 weeks). Twenty-one patients treated with at least 2 cycles of second-line MP had measurable disease, and, of these, 2 (10%) patients had an objective partial response, 1 of whom also had a PSA response. The median time to PSA progression on MP was 2.3 months, and the median duration of PSA response for responders was 5.9 months (range, 2.7–8.2 months). Three responders discontinued treatment because of toxicity (minor decreases in cardiac ejection fraction did not meet criteria for an adverse event according to National Cancer Institute's Common Toxicity Criteria v2.0 in 2 patients; thrombocytopenia occurred in 1 patient), 4 discontinued for progressive disease, and 1 died without disease progression.

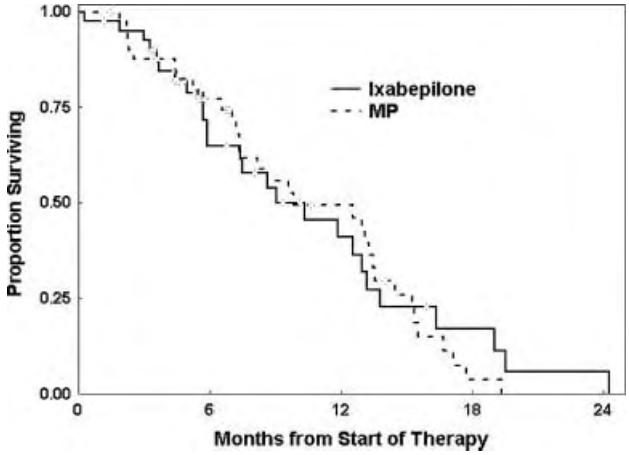

FIGURE 2. Overall survival.

An exploratory analysis of the impact of initial response to front-line taxane-based therapy on response to second-line therapy was performed. Stratified by second-line treatment, there was a significantly greater response to second-line therapy among patients who previously responded to taxane therapy (Mantel-Haenszel test: $P = .0004$).[10] The association was similar for both second-line treatment groups (test of homogeneity: $P = 0.87$). Among patients with a prior PSA response to taxane chemotherapy, 36% (5 of 14; 95% CI, 13-65) responded to ixabepilone and 35% (7 of 20; 95% CI, 5-59) responded to MP. In patients without prior PSA response to taxane-chemotherapy, 4% (1 of 26; 95% CI, 0-20) of patients responded to ixabepilone, and 5% (1 of 21; 95% CI, 0-24) responded to MP.

Survival

Evaluation of survival by treatment is complicated by the finding that 56% of patients received the alternate therapy on crossover. However, the median survival for each arm was 10.4 months for ixabepilone and 9.8 months for MP. (Fig. 2) The median overall survival for the entire study was 9.8 months., and did not show differences based on prior taxane response.

Potential disease features predictive of survival from the start of second-line therapy were evaluated in patients enrolled on this study in an exploratory analysis. When the entire study sample was dichotomized at the median baseline value, a significantly prolonged survival was observed for decreased LDH (≤270 vs >270), decreased alkaline phosphatase (≤130 vs >130) and increased hemoglobin (≤12 vs >12) ($P = .007$, .003, and .01, respectively).

TABLE 3
Maximal Grade 3-4 Hematologic Toxicity

	Ixabepilone		MP	
	2nd-Line, n = 41	3rd-Line, n = 29	2nd-Line, n = 41	3rd-Line, n = 16
	No. (%)	No. (%)	No. (%)	No. (%)
Anemia	4 (10)	2 (7)	1 (2)	—
Neutropenia	22 (54)	10 (33)	26 (63)	10 (63)
Febrile neutropenia	2 (5)*	2 (7)	4 (10)	—
Thrombocytopenia	3 (7)	3 (10)	1 (2)	1 (6)

* 1 patient died of neutropenic sepsis.

TABLE 4
Maximal Grade 3-4 Treatment-Related Non-Hematologic Toxicity

	Ixabepilone				MP			
	2nd-Line, n = 41		3rd-Line, n = 30		2nd-Line, n = 41		3rd-Line, n = 16	
Grade	3	4	3	4	3	4	3	4
GI								
Nausea/vomiting	2		1				2	
Anorexia	2							
Stomatis/pharyngitis	3		1					
Diarrhea			1					
Constipation					1			
Dehydration	1		3					
Hepatic	2		1		4			
Hypotension			3					
Fatigue	1	1	4					
Muscle weakness	2		2					
Renal		1						
Neurologic								
Motor neuropathy	1		2					
Sensory neuropathy			1					
CNS ischemia		1						
Syncope	1							
Lightheadedness	1		1					
Mood alteration	1							
Elevated PT	3		1		2			
Metabolic								
Hypophosphatemia	1		3					
Hypoglycemia				1				
Hyperuricemia		1						
Hypercalcemia		1	1					
Hypokalemia			1					
Hypersensitivity		1	1					

The following grade 3 toxicities occurred with second-line ixabepilone in 1 patient: thrombosis, atrial arrhythmia, urinary obstruction, and chest pain.

The 3 laboratory parameters were highly correlated ($P < .002$ for all pairwise comparisons). Patients without visceral disease also achieved a significantly longer survival ($P = .02$). Categorized LDH ($\leq$270 vs $>$270) was highly associated with visceral disease ($P = .005$). There was no difference in survival due to baseline performance score, PSA, or Gleason score. When the 4 individual parameters significant to predicting survival were considered simultaneously by using Cox proportional hazard model, a decreased LDH and absence of visceral metastases emerged as significant independent predictors of prolonged survival (likelihood ratio test, $P = .0003$, .04, respectively).

Toxicity

Grade 3 or 4 neutropenia occurred in 54% and 63% of patients treated with second-line ixabepilone and MP, respectively (Table 3). Febrile neutropenia and neutropenic infection occurred in 4 patients treated with second-line MP and 3 patients treated with second-line ixabepilone (including 1 patient who died from neutropenic sepsis). Treatment-related nonhematologic toxicities observed in $\geq$5% of patients treated with second-line ixabepilone included anorexia, stomatitis, fatigue, muscle weakness, and prolonged prothrombin time (Table 4). Treatment-related nonhematologic toxicity observed in $\geq$5% of patients treated with second-line MP included prolonged prothrombin times and liver function abnormalities. Dose reduction or delay were required in 20 of 41 (49%) patients treated with second-line ixabepilone and 10 of 41 (24%) patients treated with second-line MP.

Crossover Therapy

Sixteen of 41 (39%) patients on second-line ixabepilone crossed over to MP treatment. Of the 25 patients who did not cross over to MP, 8 withdrew consent, 2 died, and 14 experienced clinically significant disease progression and/or treatment-related toxicity such that they did not cross over. Four of 15 evaluable patients who received third-line MP achieved a confirmed $\geq$50% PSA decline (27%; 95% CI, 8-55; Table 5). One of 9 (11%) patients with measurable disease and at least 2 cycles of therapy demonstrated an objective response to third-line MP in addition to a PSA response.

Thirty of 41 (73%) patients on second-line MP crossed over to ixabepilone therapy. Of the 11 patients who did not cross over to ixabepilone, 2 withdrew consent, 1 died, 1 was not eligible to continue on study because of decreased clinical status, and 7 patients experienced clinically significant disease progression and/or treatment-related toxicity such that they did not cross over. Three of 27 (11%; 95% CI, 2-29)evaluable patients achieved a confirmed $\geq$50% PSA decline to third-line ixabepilone. One of 14 (7%) patients with measurable disease and at least

TABLE 5
Response to Crossover Therapy

	3rd-Line MP, n = 16	3rd-Line ixabepilone, n = 30
PSA responses	**No. (%)**	**No. (%)**
Evaluable patients*	15	27
Confirmed PSA decline ≥50%, 95% CI	4 (27, 8-55)	3 (11, 2-29)
Unconfirmed PSA decline ≥50%	—	1 (4)
Objective disease responses		
Measurable disease	11	15
Evaluable patients*	9	14
Partial response (RECIST)	1	1

* Received at least 2 cycles.

2 cycles of therapy demonstrated both an objective and a PSA response.

None of the patients who achieved a PSA response to third-line therapy demonstrated a PSA response to second-line treatment. None of the patients who responded to third-line ixabepilone and only 1 patient who responded to third-line MP had achieved a previous response to front-line taxane chemotherapy.

DISCUSSION

This study evaluated second-line chemotherapy in TR-HRPC patients to address the question of clinical cross-resistance between taxanes, epothilones, and mitoxantrone, as well as to explore the natural history of chemotherapy-refractory HRPC. MP is the de facto community standard second-line chemotherapy for HRPC in the absence of prospective data in this setting. Therefore, determining the activity of second-line MP is important not only to understand the usefulness of this regimen as second-line chemotherapy but also to define its activity as a control arm for future second-line clinical trials. Encouraging preclinical activity in taxane-resistant model systems and substantial activity seen in front-line HRPC chemotherapy support the testing of ixabepilone in the second-line setting.

The median survival for patients with TR-HRPC has not been prospectively evaluated. In the present multicenter study, the median survival of all patients was 9.8 months from the initiation of second-line chemotherapy. As study treatments demonstrated only modest activity in this setting, this value provides a useful estimate of survival as a baseline for development of future clinical trials in this patient population.

Treatment of TR-HRPC with MP or ixabepilone demonstrated only modest activity. The PSA response proportions for MP and ixabepilone were 20% and 17%, respectively. Objective responses were infrequent (≤10% each arm). Although this study was not designed to compare the 2 regimens, the levels of activity in this study appear similar between the 2 arms. The anticancer activity of ixabepilone as measured by PSA declines and objective tumor responses contrasts with results of chemotherapy-naive HRPC trials with this drug. Although 17% of patients did experience PSA responses to ixabepilone in this study, this level of activity is not sufficient to justify further evaluation of ixabepilone in this dose and schedule as single-agent second-line HRPC chemotherapy.

Although patients were required to have progressive disease during or shortly after stopping taxane chemotherapy, 35% of ixabepilone and 49% of MP patients previously experienced a ≥50% PSA decline to first-line taxane therapy. A retrospective analysis demonstrated that patients who experienced a PSA response to prior therapy were 7-fold to 8-fold more likely to respond to either second-line regimen. On the basis of these findings, future randomized studies should stratify patients for best response to prior therapy. In addition, patients who never responded to taxane-based therapy are unlikely to respond to ixabepilone or MP, and investigational therapy should be considered. In an exploratory analysis, elevated LDH and the presence of visceral metastases appear to be independent prognostic indicators of poor overall survival in the second-line setting. These indicators should be investigated further in future second-line chemotherapy studies.

The predominant toxicities seen were hematologic in nature. MP was well tolerated, with only 1 episode of neutropenic infection. Ixabepilone treatment resulted in 1 treatment-related death from neutropenic sepsis during Cycle 1. Although nonhematologic toxicities were seen with ixabepilone, none were observed with high frequency, and no single toxicity predominated. Low rates of neurotoxicity seen in this study compared with other trials of ixabepilone may in part be explained by the requirement that all patients enrolled were required to have grade ≤1 neuropathy after taxane chemotherapy. This requirement may have selected a population less susceptible to neuropathy.

Previously, the noncross-resistance of taxanes and ixabepilone was reported in a retrospective analysis of patients treated on a randomized phase 2 trial of first-line ixabepilone with or without estramustine.[14] In that analysis of 49 patients, 51% of patients treated with second-line taxane achieved a ≥50% PSA decline. The results of the current study suggest there may be a sequence-dependent effect of epothilone and that taxane therapy that may be responsible

for the lower level of activity seen with second-line ixabepilone.

In the present study, some patients who experienced disease progression on either MP or ixabepilone and crossed over to the third-line therapy achieved third-line PSA responses. In fact, none of the patients who responded to their third-line treatment responded to their second-line therapy. This implies some non-cross-resistance between the 2 regimens.

Although substantial progress in treating HRPC has been achieved with the introduction of effective first-line chemotherapy, the identification of new agents with high activity in front-line and TR-HRPC patients remains a priority. Median survival of patients with TR-prostate cancer from the start of second-line chemotherapy remains short. Both novel biologic agents as well as novel chemotherapies must continue to be investigated to improve survival in this patient population. Stratification by prior treatment response should be incorporated into future randomized clinical trials.

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Curriculum Vitae

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EDUCATION:

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PRINCIPAL POSITIONS HELD:

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HONORS AND AWARDS:

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2001-2002	Amgen Fellowship, UCSF Division of Hematology/Oncology
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1991	Howard Hughes Undergraduate Research Fellow, Duke University
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PROFESSIONAL ORGANIZATIONS:

2003-present	Cancer and Leukemia Group B
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SERVICE TO PROFESSIONAL PUBLICATIONS:

2003-present	Ad hoc referee for Journal of Clinical Oncology, Cancer, JAMA, Journal of Urology, Cancer Chemotherapy and Pharmacology, Clinical Genitourinary Cancer

INVITED PRESENTATIONS:

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2005	ASCO/AACR Multidisciplinary Prostate Cancer Symposium, Orlando, FL
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2007	Survivor's Day, California Kidney Cancer Foundation, San Francisco, CA
2007	UCSF Cancer Center Annual Cancer Update, Squaw Creek, CA
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2006	UCSF Cancer Center Annual Cancer Update, Squaw Creek, CA
2006	Survivor's Day, Kidney Cancer Foundation, San Francisco, CA
2006	Recent Advances in Renal Cell Carcinoma, San Francisco, CA
2005	UCSF Cancer Center Annual Cancer Update, Squaw Creek, CA
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2003	UCSF Division of Hematology/Oncology Grand Rounds, San Francisco, CA
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UNIVERSITY AND PUBLIC SERVICE:

2007	Radiation Oncology Search Committee
2005-present	UCSF Comprehensive Cancer Center Protocol Review Committee
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TEACHING:

2006	Small Group leader, UCSF Medical School, "Cancer: Bench to Bedside"

2005-present Assistant Clinical Professor, UCSF School of Medicine, 64 hours per month supervision of fellows and residents in outpatient clinic
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FORMAL POSTGRADUATE COURSES:
2006-present Director of Clinical Research Module, UCSF Division of Hematology/Oncology Fellowship Core Curriculum Lecture Series.
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FELLOWS DIRECTLY SUPERVISED OR MENTORED:

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2003-2004	Jorge Garcia, MD	Urologic Oncology Fellow	Clinic Preceptor	Associate Staff, Cleveland Clinic
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RESEARCH AND CREATIVE ACTIVITIES:
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1. W81XWH-05-1-0175; PC041220 1/1/05-present
DOD Prostate Cancer Research Program
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"Gene expression analysis of circulating hormone refractory prostate cancer micrometastases."

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Clinical Trial Development Award
"Phase I/II trial of epothilone analog ixabepilone, mitoxantrone, and prednisone in hormone refractory prostate cancer patients previously treated with chemotherapy"

PUBLICATIONS
ORIGINAL RESEARCH:
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2. **Rosenberg J**, Small EJ. Second-line chemotherapy for hormone refractory prostate cancer: Has the time come? Clin Prostate Cancer 3(2): 122-4, 2004.

3. **Rosenberg J**, Carroll P, Small EJ. Update on chemotherapy for advanced bladder cancer. J Urol 174: 14-20, 2005.

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b. **Rosenberg JE**. Activity of first- or second-line chemotherapy with docetaxel or mitoxantrone in patients with hormone-refractory prostate cancer. Am J Hematol/Oncol 5: 558-60, 2006.

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